

FIG. 1A

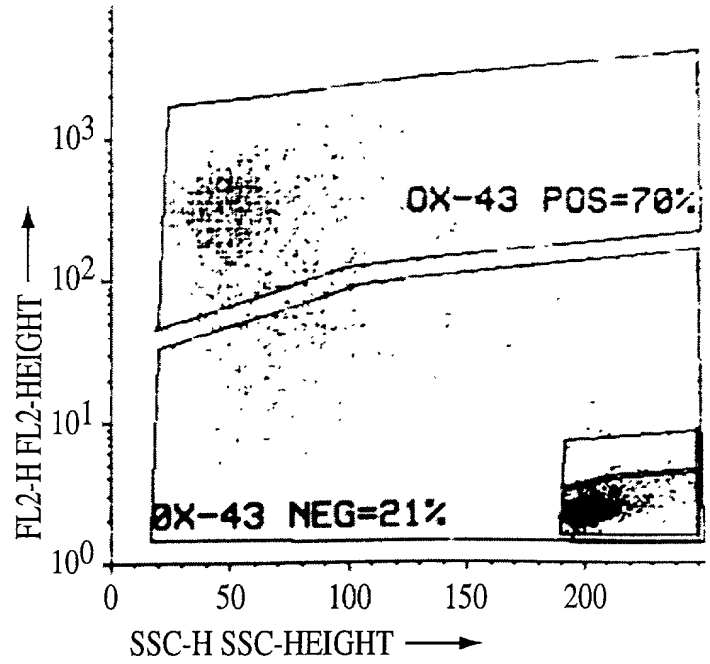


FIG. 1B

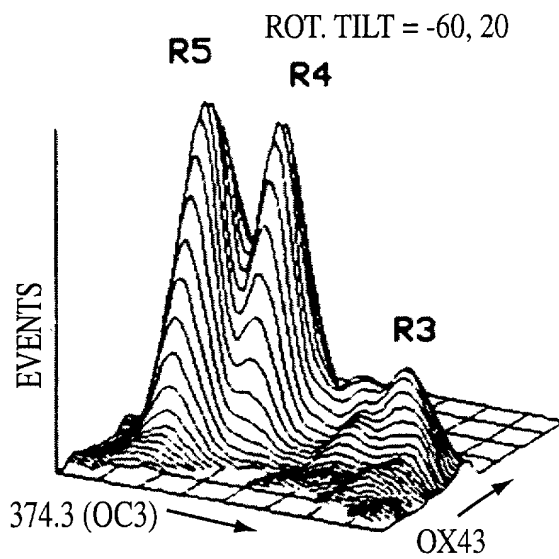


FIG. 1C

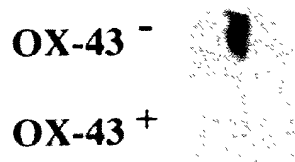


FIG. 2A

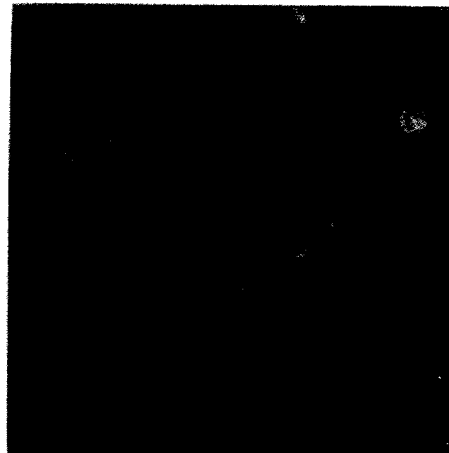


FIG. 2C

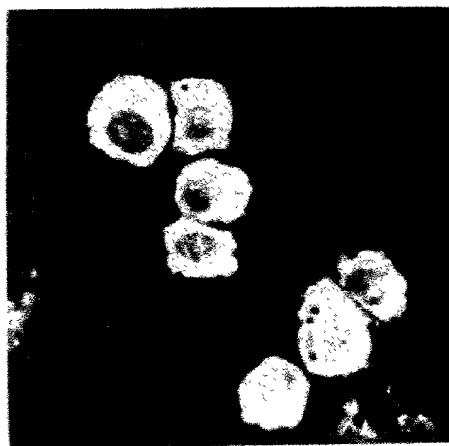


FIG. 2B

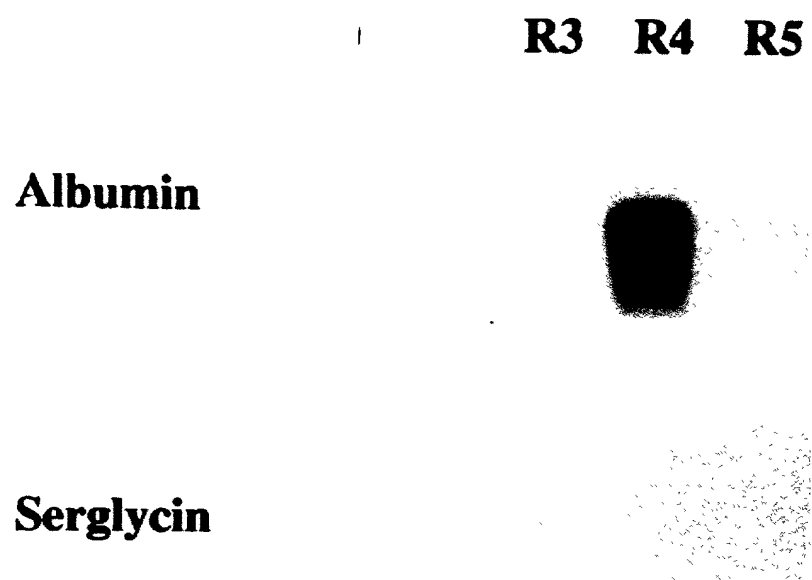


FIG. 3

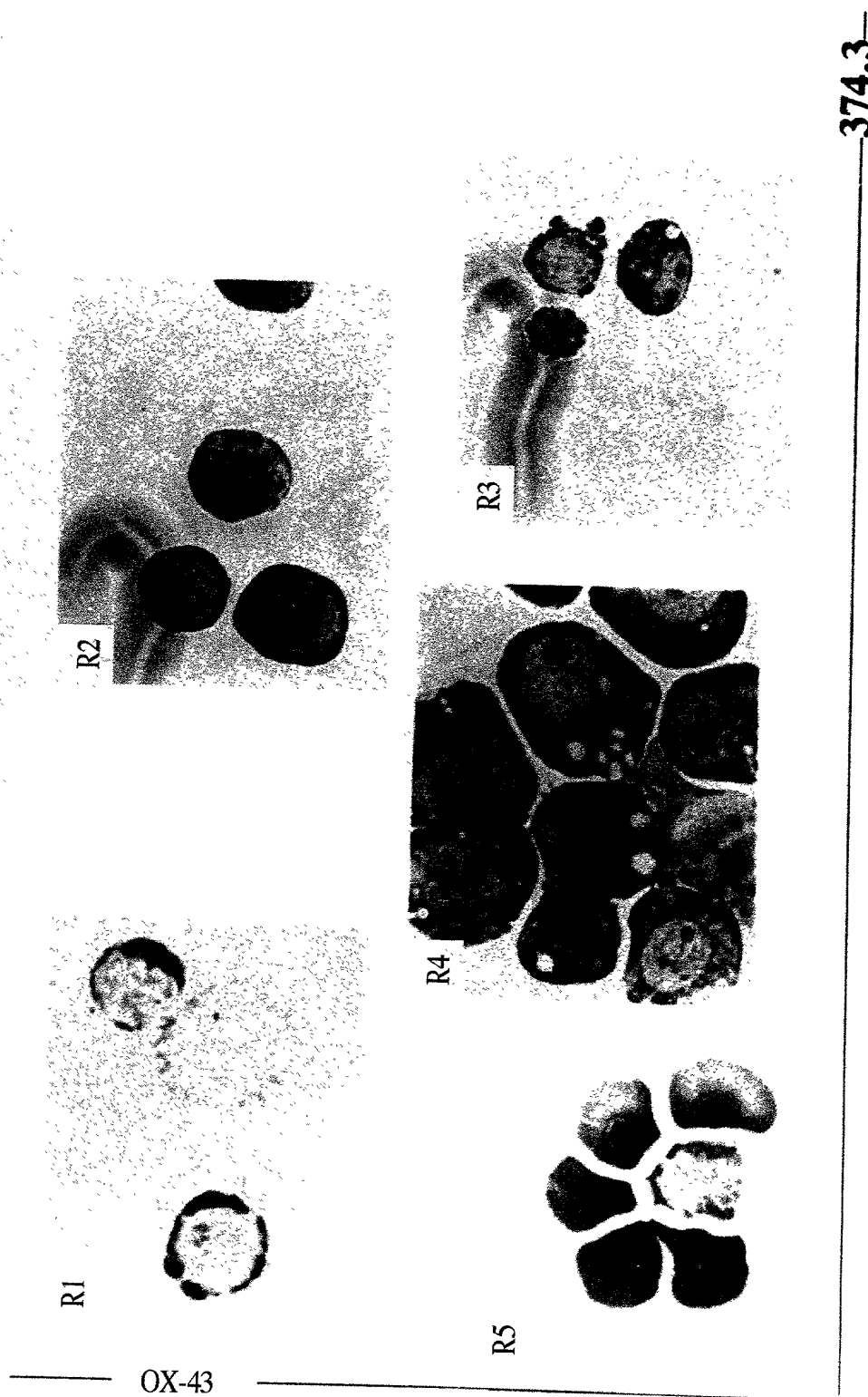


FIG. 4

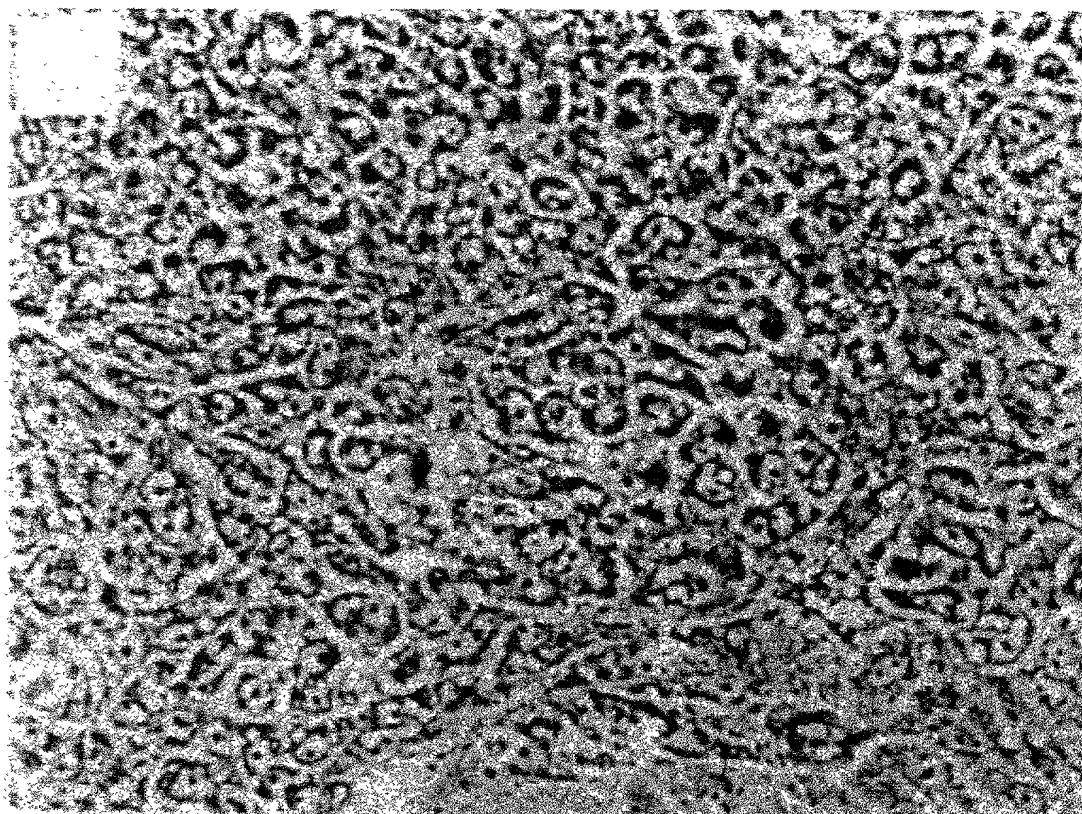


FIG. 5A



FIG. 5B



FIG. 5C

FLOW DIAGRAM OF HEPATOBLAST ENRICHMENT

LIVERS (8-9 mgs)

↓ DIRSPERSION WITH EGTA AND THEN COLLAGENASE

SINGLE CELL SUSPENSION PREPARATION: COLLAGENASE,
EGTA, 4° C↓ 10^7 CELLS/8 mgs LIVER↓ 3.2 ± 1.3 % ARE ALB⁺↓ 2.5 ± 0.7 % ARE AFP⁺↓ 87.9 ± 2.5 % ARE OX43/44⁺

PANNING

RED BLOOD CELL PANNING (2X)

↓ 29 ± 5 % OF CELLS REMAIN↓ 9.5 ± 1.2 % ARE ALB⁺↓ 9.8 ± 0.9 % ARE AFP⁺↓ 80.4 ± 3.9 % ARE OX43/OX44⁺

OX-43/OX-44 PANNING (MYELOID AND ENDOTHELIAL CELLS)

↓ 16 ± 4 % OF CELLS REMAIN↓ 14.8 ± 3.6 % ARE ALB⁺↓ 14.9 ± 2.5 % ARE AFP⁺↓ 69 ± 10 % ARE OX43/OX44⁺

FLUORESCENCE ACTIVATED CELL SORTING

NEGATIVELY SORT FOR CONTAMINANT CELL POPULATIONS:

OX-43(CD)/OX-44(CD37)⁺ CELLS = PRECURSORS AND MATURE FORMS OF HEMOPOIETIC CELLS
(MYELOID, ERYTHROID) AND ENDOTHELIAL CELLSOF REMAINING CELLS (OX-43⁻ + OX-44⁻ CELLS), SORT FOR CELLS VARYING IN OC.3
EXPRESSION AND GRANULARITY:OX-43(CD)/OX-44(CD37)⁺ CELLS = MOSTLY HEPATIC PRECURSORS, SOME RESIDUAL HEMOPOEITIC
CELL CONTAMINANTS, STROMAL CELLSOC.3⁻, GRANULAR CELLS = COMMITTED BILE DUCT PRECURSORS (AFP⁺, ALB⁻)OC.3⁻, GRANULAR CELLS = COMMITTED HEPATOCYTE PRECURSORS (AFP⁺, ALB⁺⁺⁺)OC.3⁺, AGRANULAR CELLS = EARLY HEPATOBLASTS (AFP⁺⁺⁺, ALBUMIN⁺ AND CK 19⁻)

FIG. 6

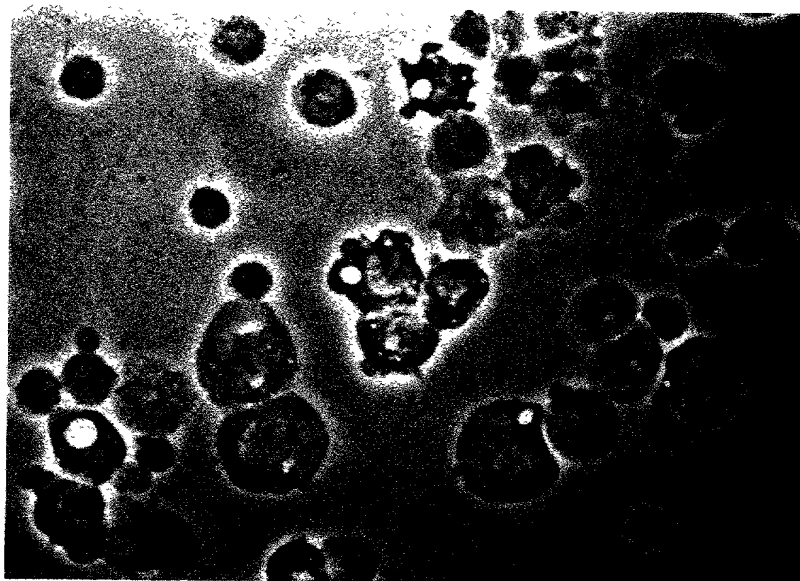


FIG. 7A



FIG. 7B

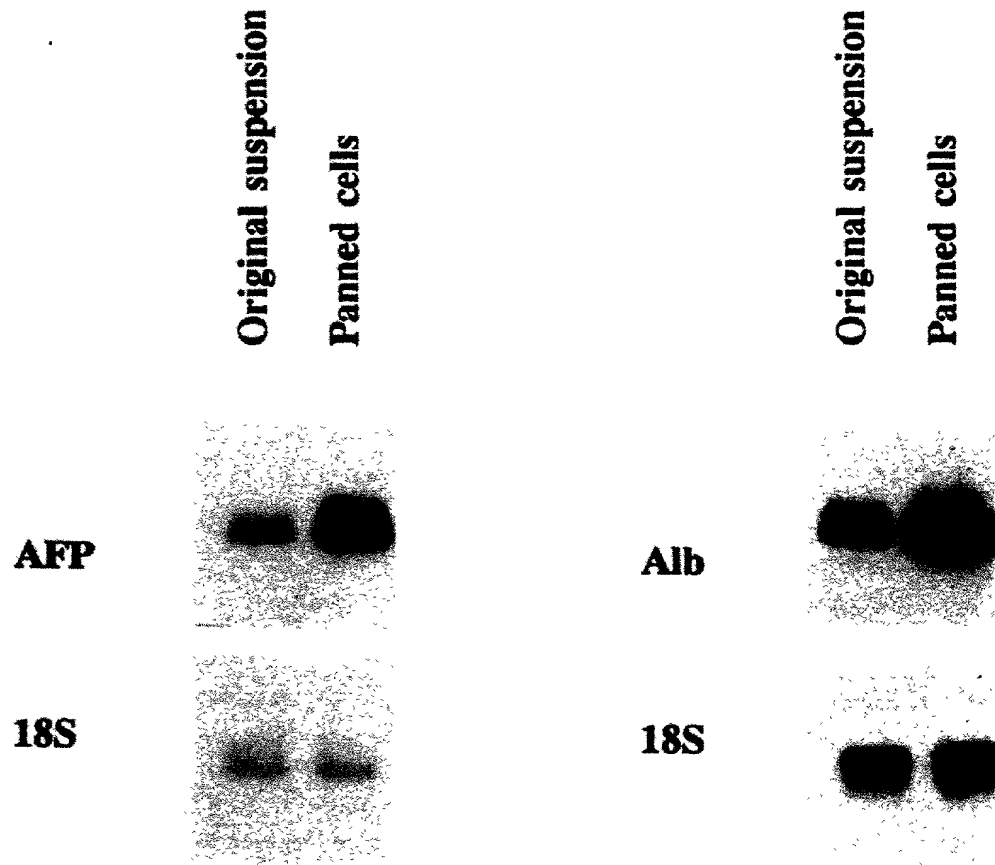


FIG. 8

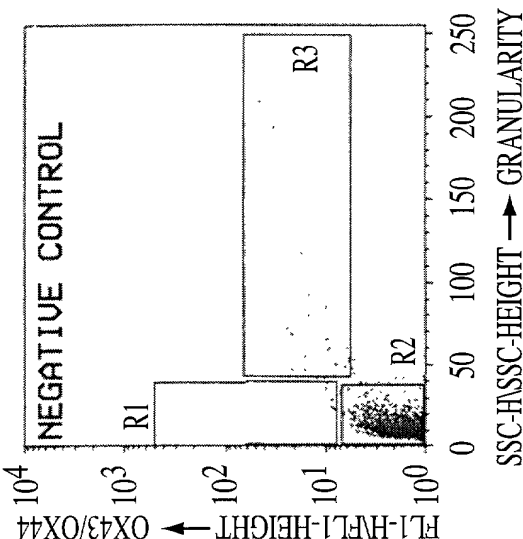


FIG. 9A

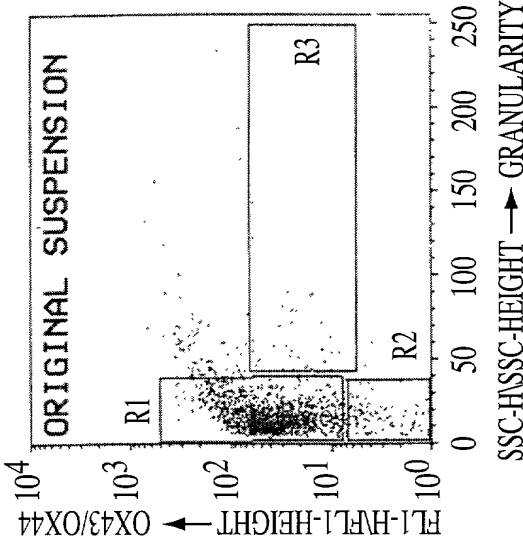


FIG. 9B

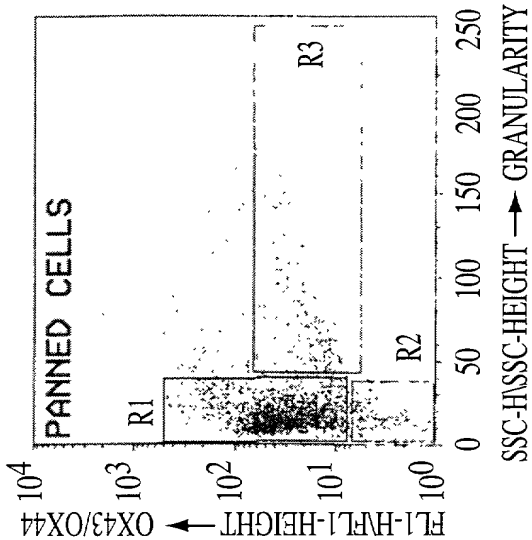


FIG. 9C

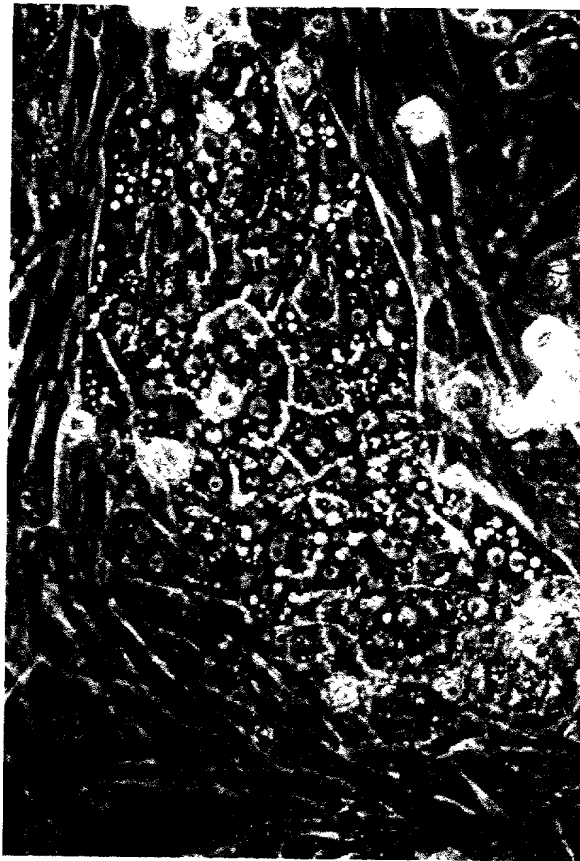


FIG. 10



FIG. 11
